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Flavonol glycosides from whole cottonseed by-product

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Abstract

Cottonseeds are fed to high-producing dairy cows as a source of fat and highly-digestible fibre. Seven flavonol glycosides have been identified from whole cottonseed by-product. Their structures were established as quercetin 3-*O*-{β-D-apiofuranosyl-(1 → 2)-[α-L-rhamnopyranosyl-(1 → 6)]-β-D-glucopyranoside} (**1**), kaempferol 3-*O*-{β-D-apiofuranosyl-(1 → 2)-[α-L-rhamnopyranosyl-(1 → 6)]-β-D-glucopyranoside} (**2**), quercetin 3-*O*-[β-D-apiofuranosyl-(1 → 2)-β-D-glucopyranoside] (**3**), quercetin 3-*O*-β-D-glucopyranoside (**4**), kaempferol 3-*O*-[α-L-rhamnopyranosyl-(1 → 6)-β-D-glucopyranoside] (**5**), quercetin 3-*O*-[α-L-rhamnopyranosyl-(1 → 6)-β-D-glucopyranoside] (**6**), and kaempferol 3-*O*-α-L-rhamnopyranoside (**7**). Gallic acid (**8**) and 3,4-dihydroxybenzoic acid (**9**) were also found. All structures were elucidated by ESI-MS and NMR spectroscopic methods. Total polyphenols were assayed by the Folin–Ciocalteu method.

Keywords: Whole cottonseed; By-products; Flavonol glycosides; 1D and 2D NMR; Feedstuff

1. Introduction

Agricultural processing yields many by-products that have significant feeding value for livestock. Because of their increasing market value as feed ingredients, many producers of such materials now consider them to be "co-products" rather than "by-products." Agricultural co-products that are sometimes available at attractive prices include soy hulls, wheat midds, whole cottonseed, wet and dry corn gluten feed, wet and dried brewers grains, dried distillers grains, poultry litter and many others.

Whole cottonseed (WCS) is the unprocessed and unadulterated oilseed which has been separated from cotton fibre. Delinted cottonseed is the unprocessed and unadulterated oilseed which has been separated from cotton fibre with less than 5% retained lint. Cottonseeds are fed to high-producing dairy cows as a source of fat and highly-digestible fibre. They are also used as a forage replacer. WCS is an excellent source of energy, protein, and effective fibre WCS dry matter is high in fat (200 g/kg), crude protein (CP, 230 g/kg) and neutral detergent fibre (NDF, 440 g/kg); this is reflected in its high energy content (9.2 MJ of net energy for lactation, NEL). The CP:NEL ratio (about 1 g CP to 40 kJ NEL) makes WCS a favourable supplement which meets the combined energy and CP requirements for high-producing dairy cows (National Research Council, 1989). The addition of WCS to the diet of lactating cows during hot weather significantly increased milk yield, milk fat content and yield, and blood plasma triglyceride, cholesterol and phospholipid concentrations (Belibasakis & Tsirgogianni, 1995).

Secondary constituents in whole cottonseed have been studied because some components, including terpenoid phytoalexins (Stipanovic, Bell, & Howell, 1975), have been blamed for anti-nutritive or toxic effects when the seeds were tested as animal feed (Gambill & Humphrey, 1993; Skutches, Herman, & Smith, 1974). Gossypol is the main anti-nutrient limiting the use of cottonseed in monogastric animals and humans, where it acts by reducing the oxygen-carrying capacity of the blood and results in shortness of breath and of edema of the lungs (Alford, Liepa, & Vanbe-

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ber, 1996). The US Food and Drug Administration sets a limit for free gossypol at 450 mg/kg in human food products and ingredients, and the FAO and WHO set the maximum limitation at 12,000 mg/kg of total gossypol (Lusas & Jividen, 1987).

Information about other phenolic compounds, such as flavonoids, in WCS is scarce and only recently did Zhang, Yang, Zhao, Luan, and Ke (2001) find that glandless cotton seed contains five flavonol glycosides, including a new apiosyl derivative. Flavonoids have been recognized as active principles, showing structure-dependent physiological activity (antioxidant, cancer-preventing, and antimicrobial) (Packer, Hiramatsu, & Yoshikawa, 1999), and their occurrence in food and feed is highly desirable. Owing to the importance of whole cottonseed as a feedstuff, for its high fat and protein contents, we have undertaken a systematic investigation of its secondary metabolites. Particular emphasis was placed on flavonoids that have been shown to possess a range of biological activity which could be beneficial to animal health and potentially could improve flavour and shelf life of farm products (Aerts, Barry, & McNabb, 1999; Simpson & Uri, 1956). Phenolic constituents, in particular, have been shown to be effective free radical-scavengers and antioxidants which are central to the maintenance of homeostasis in biological systems (Ames, Shigenaga, & Hagen, 1993; Torel, Cillard, & Cillard, 1986). Proanthocyanidins and flavonoids have been reported to exhibit antimicrobial (Kabuki et al., 2000; Marwan & Nagel, 1986; Stavric, 1994) and anti-inflammatory activities (Kakegawa et al., 1985; Shoskes, 1998).

As these phenolic compounds are increasingly being regarded as contributing to animal health and productivity, it is important that considerations of the nutritive value of forages should include, not only the primary metabolites, but also the phenolic compounds which they contain.

2. Materials and methods

2.1. Plant material

Whole cottonseed is a by-product of cotton production and acreage is expanding in the north of Italy. Whole cottonseed (WCS) was furnished by Cereal Comm Feed Company (Brescia, Italy).

2.2. Spectroscopic apparatus

UV spectra were recorded on a Shimadzu UV-2101PC, UV–Vis scanning spectrophotometer. A Bruker DRX-600 NMR spectrometer, operating at 599.19 MHz for ^{1}H and at 150.86 MHz for ^{13}C, using the UXNMR software package, was used for NMR experiments; chemical shifts are expressed in δ (parts per million) referred to the solvent peaks δ_H 3.34 and δ_C 49.0 for CD_3OD; coupling constants, J, are in Hertz. DEPT ^{13}C, 1D-TOCSY, ^{1}H–^{1}H DQF-COSY, ^{1}H–^{13}C HSQC, and HMBC NMR experiments were carried out using conventional pulse sequences, as described in the literature. Electrospray ionization mass spectrometry (ESI-MS) was performed using a Finnigan LC-Q Advantage instrument from Thermoquest (San Jose, CA) equipped with Excalibur software. Samples were dissolved in MeOH and infused in the ESI source by using a syringe pump; the flow rate was 3 μl/min. The capillary voltage was 5 V, the spray voltage 5 kV, and the tube lens offset 35 V. The capillary temperature was 220 °C and the data were acquired in the MS1 and MS/MS scanning modes. The scan range was m/z 150–900 and for the MS/MS scanning mode, the percentage of collision energy was 30%. GC analyses were performed using a Chrompack (Middelburg, The Netherlands) model 9001 gas chromatograph with a data-handling system and FID.

2.3. Extraction and isolation procedure of compounds 1–9

The dried and powdered WCS (405 g) was defatted with hexane and $CHCl_3$ and then extracted with MeOH to give 19 g of residue. The MeOH extract was partitioned between *n*-BuOH and H_2O to afford a *n*-BuOH soluble portion (5.8 g) which was chromatographed twice on a Sephadex LH 20 CC (Pharmacia, Uppsala, Sweden) (1 m × 3 cm i.d.) column with flow rate of 0.5 ml/min; 90 fractions of 8 ml were collected. After TLC analysis (Si-gel, *n*-BuOH–AcOH–H_2O 65:15:25, $CHCl_3$–MeOH–H_2O 70:30:3), fractions with similar R_f were combined, giving four major fractions (I–IV) which were further purified by HPLC on a Waters 590 series pumping system equipped with a Waters R401 refractive index detector, a Waters μ-Bondapak C-18, 10 μm (7.8 × 300 mm, flow rate 2.5 ml/min) column and a U6K injector. Fraction II (450 mg) was purified using MeOH–H_2O (40:60) as the eluent, to yield pure flavonol glycosides **1** (30.0 mg, $R_t = 11$ min), **2** (19.0 mg, $R_t = 12$ min), **3** (42.0 mg, $R_t = 18$ min), **4** (28.1 mg, $R_t = 21$ min), **5** (41.3 mg, $R_t = 22$ min), **6** (22.4 mg, $R_t = 28$ min), **7** (29.0 mg, $R_t = 31$ min). Fraction III (205 mg) was purified using MeOH–H_2O (30:70) to yield pure compounds **8** (101.5 mg, $R_t = 10.1$ min) and **9** (78.0 mg, $R_t = 11.8$ min).

2.4. Acid hydrolysis of compounds 1 and 2

A solution of each compound (3.0 mg) in 6% aqueous HCl (3.5 ml) was refluxed for 2 h. The reaction mixture was diluted with H_2O and then extracted with EtOAc. The resulting aglycones were identified by their ^{1}H NMR spectra.

2.5. Methanolysis of compounds 1 and 2

Compounds (1.0 mg of each) were heated in a sealed vial for 24 h at 80 °C in 2% MeOH–HCl (2 ml). After MeOH and HCl distillation in a N_2 stream, Ag_2CO_3 and MeOH were added until CO_2 production stopped. The centrifugate was dried over P_2O_5. The resulting monosaccharides were treated with TRISIL-Z (Pierce) and analysed by GC.